BARD Final Report IS-4919-16 R

Systematic analyses of the roles of *Solanum Lycopersicum* ABA receptors in environmental stress and development

Mosquna, A. The Hebrew University of Jerusalem
Cutler, S. University of California, Riverside

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Three year research project

Drought and other abiotic stresses have major negative effects on agricultural productivity. The plant hormone abscisic acid (ABA) regulates many responses to environmental stresses and can be used to improve crop performance under stress. ABA levels rise in response to diverse abiotic stresses to coordinate physiological and metabolic responses that help plants survive stressful environments. In all land plants, ABA receptors are responsible for initiating a signaling cascade that leads to stomata closure, growth arrest and large-scale changes in transcript levels required for stress tolerance. We wanted to test the meaning of root derived ABA signaling in drying soil on water balance. To this end we generated transgenic tomato lines in which ABA signaling is initiated by a synthetic agonist- mandipropamid. Initial study using a Series of grafting experiments indicate that that root ABA signaling has no effect on the immediate regulation of stomata aperture. Once concluded, these experiments will enable us to systematically dissect the physiological role of root-shoot interaction in maintaining the water balance in plants and provide new tools for targeted improvement of abiotic stress tolerance in crop plants.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Reviewed	1	1	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Postdoctoral Fellow	Sun	Yufei	HUJI	Israel
Ph.D. Student	sterlin	yelena	HUJI	Israel
Ph.D. Student	Pri Tal	Oded	HUJI	Israel
M.Sc. Student	Wseglass	Gil	HUJI	Israel
Ph.D. Student	Zimran	Gil	HUJI	Israel
Postdoctoral Fellow	Park	Sang	UCR	USA

Contribution of Collaboration.

The collaborative effort was mainly to discuss the experimental procedures and crossing program for efficiently move forward to the desired transgenic lines.

Additionally the two parties met in France during 2019 to discuss results, future BARD applications and the manuscript preparation.

Drought and other abiotic stresses have major negative effects on agricultural productivity. Additionally, activation of plant stress response comes at a physiological cost that is translated to yield loss. The essence of this BARD proposal was to moderately activate stress signaling to achieve maximum stress response with minimal yield drag. To this end we generated the genetic tools to activate ABA signaling in spatial, temporal and intensity. In practical terms we can now activate tomato ABA stress response by a single receptor (instead of 15) or by synthetic agonist.

During the last years, we generated an ABA deficient *sitiens* mutant on M82 background. Additionally, in accordance with the original plan, we generated transgenic plants transformed with constructs harboring constitutive active (CA) tomato PYLs under the regulation of their native promoters. PCR confirmed or protein expressing T_0 plants were crossed with T_1 heterozygote *sitiens* mutant and F_1 hybrid seeds were collected (Table 1). We also produced transgenic plants constitutively expressing an engineered ABA receptor - $PYR1^{MANDI}$ (Park et al., 2015).

CA gene	Independent lines	PCR based confirmation	Protein expression based Western blot	Crossed with M82 *sitiens*
SlPYL10 Solyc03g007310	16	not tested	11⁄16	10⁄11
SlPYL8 Solyc06g061180	7	not tested	7⁄7	7⁄7
SlPYL15 Solyc05g052420	8	8⁄8	0⁄8	2⁄8
SlPYL6 Solyc06g050500	9	9⁄9	0⁄9	0⁄9
SlPYL3 Solyc01g095700	11	not tested	3⁄11	3⁄3
SlPYL11 Solyc10g076410	1	not tested	0⁄1	0⁄1
SlPYL9 Solyc09g015380	4	not tested	2⁄4	4⁄4
SlPYL1 Solyc08g082180	3	2⁄3	0⁄3	3⁄3

Table 1. Summary of T_0 plants recovered from tissue culture after transformation with binary constructs harboring CA tomato PYLs. Plants were subjected to PCR and/or protein expression analysis. Some of the plants were crossed with M82 *sitiens* mutant and F1 hybrid seeds recovered.

During the 2nd year, we shifted the focus to 35S::$PYR1^{MANDI}$ transgenic tomato plants, as a more feasible project considering the remaining funding period. In this genetic background the activation of ABA signaling can be achieved by application of synthetic agonist mandipropamid. We utilized the above-mentioned genetic material to understand the contribution of root ABA signaling on the overall plant stress response. To this end, we had to experimentally uncouple root and shoot with respect to ABA signaling and biosynthesis. We, therefore, initiated the following crossing program: four independent T_0 35S::$PYR1^{MANDI}$ expressing plants were crossed with T_1 heterozygote *sitiens* mutant. F_1 hybrids harboring both the transgene and the *sitiens* mutation were selfed to be screened for $PYR1^{MANDI}$/ $PYR1^{MANDI}$ and $PYR1^{MANDI}$/ $PYR1^{MANDI}$; *sit*$^{-}$/ *sit*$^{-}$ genotypes. Grafting combinations of these lines, together with *sit*$^{-}$/ *sit*$^{-}$ and wild type, will be used to investigate the role of root ABA signaling cascade in shoot ABA induced changes under stress in the upcoming months (Fig 1).

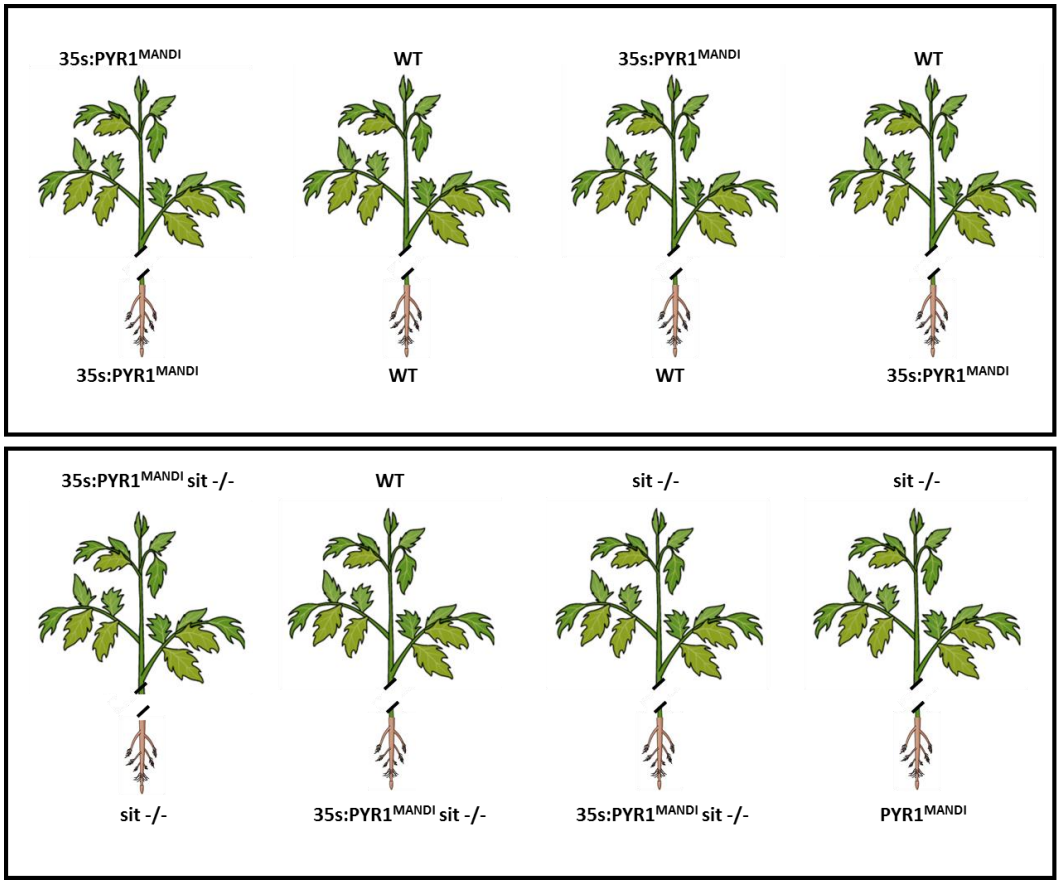

Figure 1. Evaluating the contribution of root ABA signaling to shoot stress response. The figure depicts the graft types to be made.

As a preliminary experiment, we verified the mobility of mandipropamid via the plants vascular system. To this end, segregating T_1 progeny of 35S::PYR1^{MANDI} plants were watered with either water, ABA or mandipropamid and the response was monitored by a thermograph. Due to the thermal increase observed in all transgenic plants watered with mandipropamid (Figure 2C; 7-9, 13-16 and 18-23), we conclude that mandipropamid is transported via the xylem. This observation will be further evaluated by mass spectrometry. Figure 2E demonstrates that segregating T_1 35S::PYR1^{MANDI} seedlings responding to mandipropamid (7-9, 13-16 and 18-23) do indeed harbor the *35S::PYR1^{MANDI}* cassette.

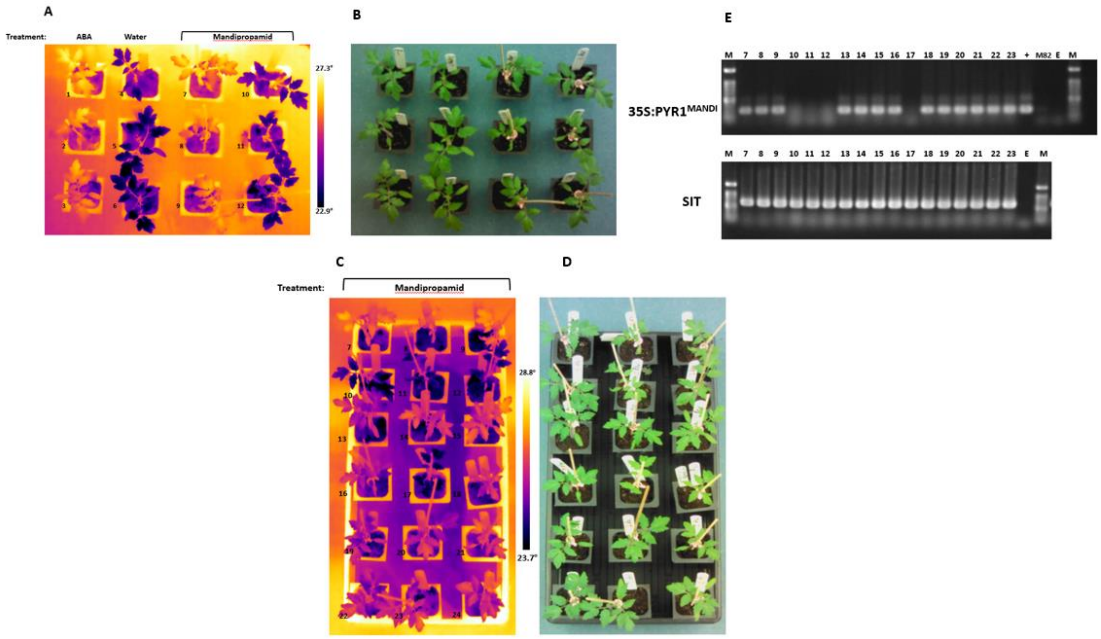

Figure 2. Thermographs of segregating T_1 35S:PYR1^{MANDI} seedlings. (A) Seedlings were watered with ABA [10µM] (positive control), water (negative control), or mandipropamid [10µM] and images taken two days post treatment. Mandipropamid induces a thermal signature in transgenic (7-9) in comparison to null segregating siblings (10-12). Increase temperature is a proxy of stomata closure and reduction of transpiration. (B) A white light image. (C) All Seedlings were watered with mandipropamid [10µM] and image taken after 2 days. Mandipropamid induces a thermal signature in transgenic (7-9, 13-16, 18-23) in comparison to null segregating siblings (10-12, 17). (D) A white light image. (E) Upper gel: *35S::PYR1^{MANDI}* specific PCR analysis for confirmation of mandipropamid responsive plants being transgenic. PCR product is 260bp long. M - gene ruler DNA ladder mix; 7-23, T_1 PYR1^{MANDI} seedlings watered with mandipropamid; (+) - binary construct used for transformation (positive control); M82 – wild type non-transgenic tomato plant (negative control), E - H_2O, water. Lower gel: *SIT* (*sitiens*) PCR – internal control.

We next addressed the question of whether stimulation of root ABA signaling affects shoot transpiration. To this end, we generated reciprocal grafts of T_2 35S::PYR1^{MANDI} homozygotes and WT tomato plants, thus confining stimulation of ABA signaling pathway to either the root or the shoot (Figure 1 top panel). Non-grafted 35S::PYR1^{MANDI}, as well as self-grafted 35S::PYR1^{MANDI} and self-grafted WT plants served as controls. Thermograph images of acclimated plants as well as controls were taken seven days after grafting (Figure 3A). Soil of the plants was then saturated with 10uM Mandipropamid solution and thermograph images taken 48hr later (Figure 3B, 3C and 3D). While non-grafted 35S::PYR1^{MANDI}, self-grafted 35S::PYR1^{MANDI} and 35S::PYR1^{MANDI} scion grafted to WT stocks demonstrated thermal increase after treatment with mandipropamid (Figure 3A versus 3B and 3C), self-grafted WT and WT scion grafted to 35S::PYR1^{MANDI} stocks remained unaffected. Our data suggest that activation of root ABA signaling is not a sufficient factor to induce stomatal closure. To further validate this hypothesis we will quantify this phenomena using the lysimeters system. Additionally the root ABA response will be verified by transcriptional activation.

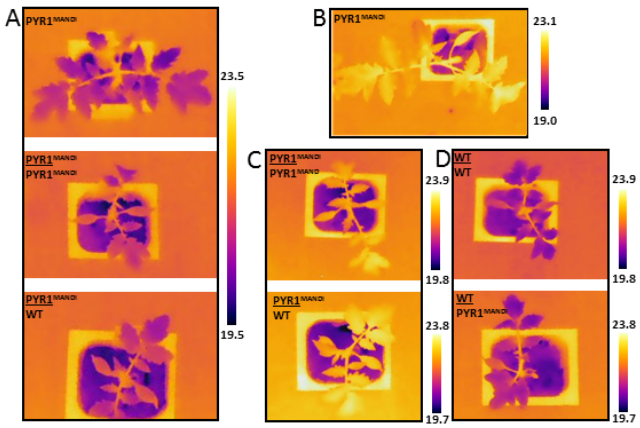

Figure 3. Change in leaf temperature in reciprocal grafts of WT and transgenic 35S::PYR1^{MANDI} tomato plants. Genotypes of scions are shown above the genotypes of the stocks. (A) Two real leaves 35S::PYR1^{MANDI} tomato seedlings were either grafted on oneself or grafted upon same age WT stocks and imaged by thermography seven days later. Non-grafted plants were used as treatment control (A and B top panels). (B-C) Plants were watered with 10µM Mandipropamid seven days post grafting and imaged by thermography 48 h after application. Leaf warming is a consequence of reduced transpiration. (D) Grafts of WT tomatoes either grafted on oneself or grafted upon 35S::PYR1^{MANDI} were similarly treated with mandopropamid.

References

Park, S.Y., Peterson, F.C., **Mosquna, A.**, Yao, J., Volkman, B.F., and **Cutler, S.R**. (2015). Agrochemical control of plant water use using engineered abscisic acid receptors. Nature 520:545-548.

Changes to original research Plan

Due difficulties in the transformation of the RR tomato cultivar and shortage of time, we did not had the chance to phenotype the *sit* mutant expressing the constitutive ABA receptors thus we shifted our efforts to analyze the transgenic plant suggested in our backup plan (1st year report), we express mandipropamid activated ABA receptor (Park et al., 2015), driven by a 35S or stomata specific promoter and tested the physiological meaning of ABA root signaling for transpiration and stomata regulation.

Park, S.Y., Peterson, F.C., **Mosquna, A.**, Yao, J., Volkman, B.F., and **Cutler, S.R**. (2015). Agrochemical control of plant water use using engineered abscisic acid receptors. Nature 520:545-548.

Publications for Project IS-4919-16 R

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Published	Reviewed	*Gil Wiseglass, Oded Pri-Tal, Assaf Mosquna*	ABA signaling components in Phelipanche aegyptiaca	***Scientific Reports***	: 2019	IS only
Published	Reviewed	*Yelena Sterlin, Oded Pri-Tal, Gil Zimran, Sang-Youl Park, Julius Ben-Ari, Jiorgos Kourelis, Inge Verstraeten,Maayan Gal, Sean R. Cutler and Assaf Mosquna*	Optimized small-molecule pull-downs define MLBP1 as anacyl-lipid-binding protein	***The Plant Journal***	: 2019	Joint